FINAL REPORT

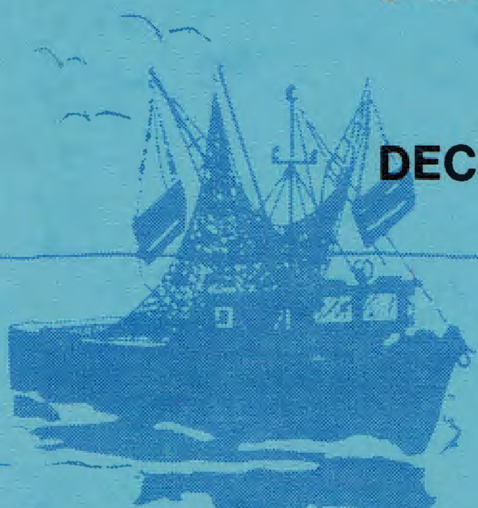
BASELINE STUDY OF THE EFFECTS OF BRINE DISPOSAL ON SELECTED MARINE ANIMALS

SURVEY No. 4

BY
TERECO CORPORATION
COLLEGE STATION, TEXAS

in collaboration with

CARBON SYSTEMS, Inc.
BATON ROUGE, LOUISIANA



DECEMBER 1980

Prepared for LOOP, Inc.
P. O. BOX 1159, Harvey, LA. 70059
Under Purchase Order 01895, as amended
A. J. HEIKAMP, Project Director

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RESULTS OF FIELD STUDY FOR LOOP, INC.

I. INTRODUCTION

SOURCES OF REPORT DATA

On 23 July 1980, TerEco Corporation forwarded a report to LOOP, Inc. covering Survey No. 3 and with some comparisons between No. 3 and Surveys No. 1 and No. 2. The June report discussed survey results obtained by TerEco and as well incorporated hydrocarbon data provided by Carbon Systems, Inc. and some water quality data transferred to TerEco by personnel of the Louisiana Wildlife and Fisheries Department and by Shilstone Engineering Testing Laboratory, Inc. with Mr. A.J. Heikamp acting as the transfer agent.

The present report deals with the results of Survey No. 4, which was mounted by TerEco between 15 and 23 November 1980. Some water quality data were supplied to TerEce by LOOP, Inc. (from Shilstone), but during this survey TerEco personnel also determined salinities and dissolved oxygen values during BOM deployment and retrieval operations. All hydrocarbon data were supplied to TerEco by Carbon Systems, Inc. Details of the hydrographic and hydrocarbon data are included as appendices, A and B, respectively. Enzyme, energy charge, and metal analyses were carried out in TerEco's laboratories. These analyses were done on animals exposed in Biotal Ocean Monitors (BOM) of both pelagic (P-BOM) and benthic (B-BOM) types placed at the survey stations.

BOM PLACEMENT AND RETRIEVAL

In Survey No. 4, eight B-BOMs and two P-BOMs were deployed among the eight stations shown in Figure 1. All BOMs were left out for three days, as shown in Table 1. In spite of moderately bad weather and zero to limited underwater visibility all BOMs were retrieved on schedule. All organisms were in good condition in contrast to Survey 3 when very low dissolved oxygen in the bottom water caused heavy mortality among test organisms.

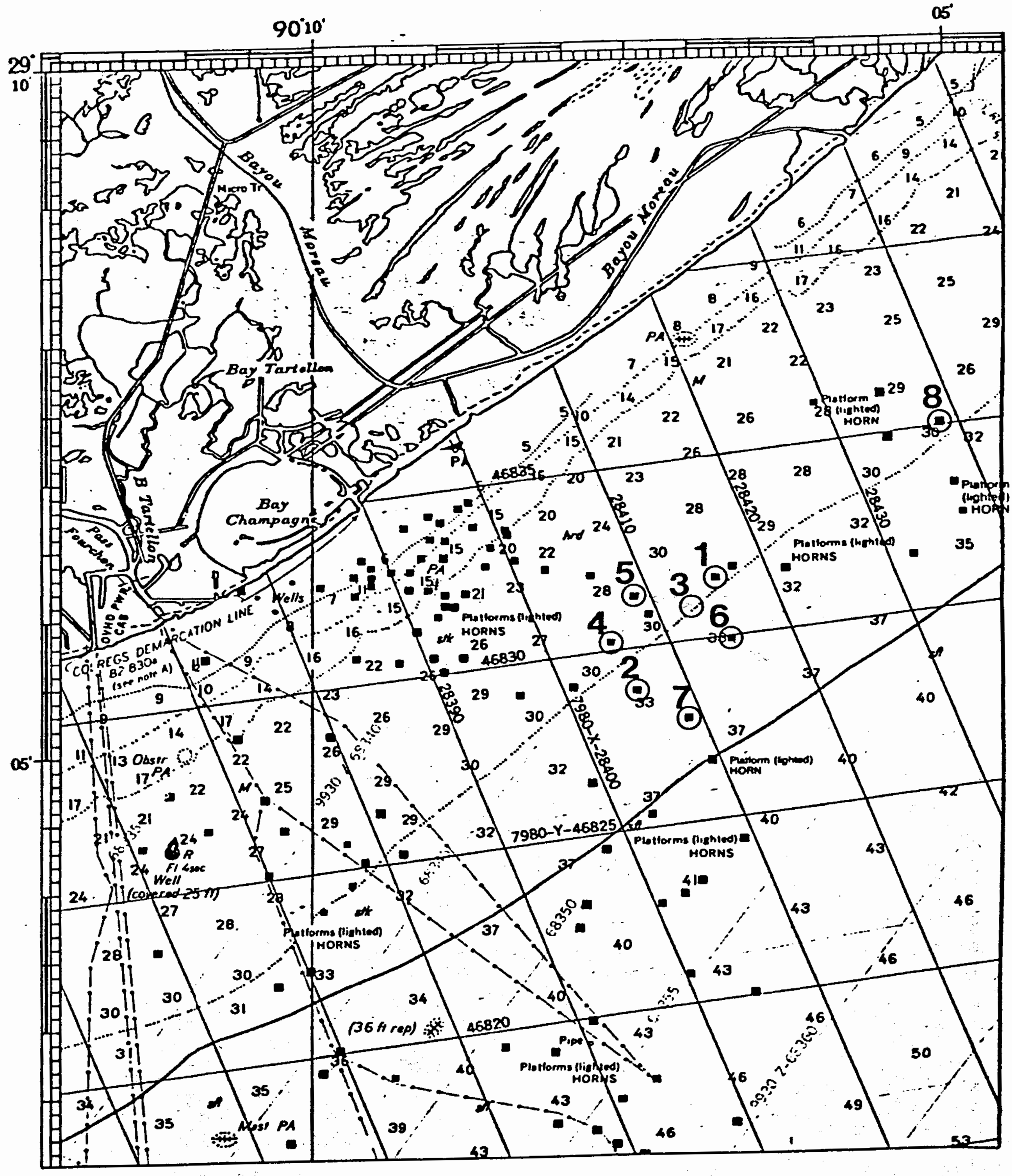

Fig. 1. Map of the eight LOOP stations off the coast of Louisiana. Note that all stations are located adjacent to an oil platform except for #3 which is the location of the brine diffuser.

TABLE 1

Deployment and Retrieval of BOMs During LOOP Survey 4
B Stands for B-BOMs and P for P-BOMs
Offshore Louisiana 1980

Date	Station Number (as in Figure 1)									
Deployment	1	2	3 (diffuser)		4	5	6	7	8 (control)	
18 November 1980	B		B,	P					B,	P
19 November 1980		B			B	B	B	B		
Retrieval										
21 November 1980	B		B,	P					B,	P
22 November 1980		B			B	B	B	B		

TYPES AND NUMBERS OF ORGANISMS UTILIZED IN TESTS

The same test organisms were used in Survey No. 4 as were used in Survey No. 3, namely, killifish (*Fundulus grandis*), grass shrimp (*Palaemonetes pugio*), brown shrimp (*Penaeus aztecus*), and oyster (*Crassostrea virginica*). The latter species was omitted from the two P-BOMs because of poor condition and insufficient numbers. The brown shrimp is never used in P-BOMs. The numbers of each species placed in the BOMs are shown in Table 2.

TABLE 2

Numbers of Test Species Placed in BOMs

Organisms	Numbers	
	P-BOMs	B-BOMS
Fundulus grandis (fish)	60	60
Palaemonetes pugio (grass shrimp)	75	75
Penaeus aztecus (brown shrimp)	0	0
Crassostrea virginica (oyster)	0	20

II. ENVIRONMENTAL MORTALITY DURING EXPOSURE

In contrast with Survey No. 3 when mortality of test organisms was very high in the B-BOMs, averaging 84 percent among the four species, mortality was very low in the B-BOMs in Survey No. 4. In fact the only serious mortality occurred among the oysters in the B-BOM at the Control Station No. 8. This can be attributed to the poor condition of the oysters when collected plus the large amount of mud found in the bottom of the unit when it was hauled aboard. Certainly all other environmental factors that were measured during the test, particularly salinity and dissolved oxygen, were wholly satisfactory. Mortality notations are shown in Table 3 along with the high mortality figures of Survey No. 3.

TABLE 3

Average Percent Mortality of Test Species
By BOM Type in Survey 4 — Compared with Survey 3
November and June 1980

Species	P-BOMs		B-BOMs	
	No. 4	No. 3	No. 4	No. 3
Fundulus grandis (fish)	0	2	2	85
Palaemonetes pugio (grass shrimp)	0	0	3	100
Penaeus aztecus (brown shrimp)	-	-	5	98
Crassostrea virginica (oyster)	-	4	20	53

The differences in mortality between the two surveys are very striking. The high mortality of Survey No. 3 and low mortality of Survey No. 4 in B-BOMs is most certainly related to dissolved oxygen and not to salinity. As is shown in the next section, dissolved oxygen had returned to normally high levels in November, whereas salinity of the bottom waters was much the same in November as in June.

III. HYDROGRAPHIC CONDITIONS DURING THE STUDY PERIOD

During Survey No. 4 the principal winds were strong and blew at first out of the northwest quadrant and just before and during BOM pickup out of the northeast quadrant. These winds, especially the shift to the NE, can account for the fact that between BOM set on November 18 and 19 and BOM pickup on November 21 and 22 the mean salinity dropped while the dissolved oxygen went up (see Tables 5 and 6).

TEMPERATURE

The mean surface water temperature during Survey No. 4 was approximately midway between the June high of 28.97°C and the December low of 17.46 (Table 4). The mean bottom water temperature on the other hand was relatively colder, being only 3.5°C above the December low but as much as 8.15°C below the June high (Table 4). Since at this time of year, the inshore water is often colder than the offshore water, it is probable that it was mixed with water from the adjacent estuaries.

TABLE 4

Mean Surface and Bottom Water Temperatures (°C) at LOOP BOM Stations During Four Surveys

	Survey 1	Survey 2	Survey 3	Survey 4
	October	December	June	November
Surface Water				
During BOM set	27.00	17.46	28.97	21.44
During BOM pickup	24.50	15.21	27.14	21.50
Bottom Water				
During BOM set	26.79	16.30	27.77	19.58
During BOM pickup	26.41	16.25	25.73	19.62

SALINITY

Although the mean surface salinity during Survey No. 4 was slightly higher than that during Survey No. 3, the bottom salinity averaged slightly lower (32.73‰ vs. 32.91‰). But as can be seen in Table 5 these salinities are well within the range of normal inshore seawater, from 32 to 34 or 35‰. The fact that in the 3-day interval that the BOMs were on station the salinity of both surface and bottom water dropped between 2 and 3 ppt indicates that the strong NE winds were pushing estuarine water offshore and pretty thoroughly mixing the water column (only a 0.15 ppt difference between surface and bottom). If we take the mean of the mean salinity values in Table 5 for the two pre-brine-discharge surveys and compare them with the values obtained from the two post-brine-discharge surveys the salinity appears to have risen no more than 2.41‰, which is about half as much as it can change within a matter of hours or days at these inshore locations (see Survey No. 2 in Table 5). Another remarkable fact is that the salinities at the Diffuser Station 3 are only slightly higher than at Control Station 8 (see Table 6).

TABLE 5

Mean Surface and Bottom Water Salinities (‰)
at LOOP BOM Stations During Four Surveys

	Survey 1	Survey 2	Survey 3	Survey 4
	October	December	June	November
Surface Water				
During BOM set	32.88	31.37	29.25	33.62
During BOM pickup	30.89	23.20	32.47	32.69
Bottom Water				
During BOM set	32.90	31.96	31.69	33.61
During BOM pickup	30.88	24.99	34.13	31.84

TABLE 6

Salinities (o/oo) of Surface and Bottom Waters at the Eight LOOP Stations During BOM Set and BOM Pickup During LOOP Survey 4

Station	BOM SET Surface	BOM SET Bottom	BOM PICKUP Surface	BOM PICKUP Bottom
Nov. 18, 1980			Nov. 21, 1980	
8 - Control	31.65	31.63	30.75	31.20
3 - Diffuser	32.61	32.61	32.03	32.15
1	32.32	32.75	31.67	32.15
Nov. 19, 1980			Nov. 22, 1980	
2	34.40	34.30	31.79	31.56
4	34.52	34.40	31.67	32.03
5	34.40	34.16	31.91	31.56
6	34.52	34.52	31.79	32.03
7	34.52	34.52	31.91	32.03

DISSOLVED OXYGEN

Dissolved oxygen (DO) concentrations during Survey No. 4 were normal in both surface and bottom waters (Table 7). There was a modest increase of little significance in DO concentrations between BOM set on November 18-19 (ave. 6.34 ppm) and BOM pickup on November 21-22 (ave. 7.08 ppm). This probably resulted from oxygenation and mixing of the water column by the high seas running in the time interval between set and pickup. As can be seen in Table 7, the DO concentrations in the bottom waters averaged well above the lows of June 1980 when such high mortality of test organisms in the B-BOMs occurred (TerEco LOOP Report No. 3, June 1980).

TABLE 7

Mean Surface and Bottom Water Dissolved Oxygen (ppm) at LOOP BOM Stations During Four Surveys

	Survey 1 October	Survey 2 December	Survey 3 June	Survey 4 November
Surface Water				
During BOM set	7.70	8.17	8.30	6.39
During BOM pickup	7.83	9.80	6.43	7.05
Bottom Water				
During BOM set	5.09	8.50	4.70	6.29
During BOM pickup	4.58	8.27	1.93	7.10

IV. CHEMICAL ANALYSIS OF ORGANISMS

TRACE METALS

Tissues used for Trace Metal Analyses

The following tissues were utilized from each of the four test species (actually 5 spp., since shrimps were a mixture of brown and white shrimps) for the metal analyses:

1. Penaeid Shrimp: muscle from tail section. Five tail sections were pooled to provide sufficient flesh for good analysis. Exoskeleton and "vein" removed.
2. *Crassostrea virginica*: adductor muscle from five individuals.
3. *Fundulus grandis*: muscle tissue and backbone from five individuals. Skin flayed off and not included.

Sample Digestion

Each tissue sample comprising 0.3 to 0.5 grams was freeze-dried for 48 hours. Each was then stirred and an aliquot weighed for digestion in a tared 180 ml spoutless electrolytic beaker. Ten ml of double distilled concentrated nitric acid was then added to each beaker. Partial digestion was accomplished in 8 hours at room temperature. After covering with a watch glass the beakers were heated in a figerglass hood until refluxing produced a clear solution. Heat was then turned off and the beakers allowed to cool at which time 3 ml of perchloric acid was added. After removing the watch glasses, the beakers were heated and the solutions evaporated to dryness. If digestion was complete, the residue was white; if not, it was yellow or green. To the latter an additional 5 ml of concentrated nitric were added and the solution refluxed and evaporated to the white residue. Beakers with no tissue but with all reagents added were treated as reagent flanks. Additionally, National Bureau of Standards reference materials (bovine liver and orchard leaves) were digested, and used as a recovery check.

One ml of nitric acid and 4 ml of distilled deionized water were added to each beaker. Low heat was applied until the white residue was dissolved. After transferring to a tared 7-dram vial, the samples were reweighed and a dilution factor calculated. Metal analyses were then determined on the solutions by means of AA spectrophotometry.

Results of Trace Metal Analyses

As was noted on page 13 of TerEco's LOOP Report No. 3, there is a marked seasonal difference in the degree to which the test organisms accumulate or unburden themselves of metals. For instance, the oyster appears to accumulate zinc and copper to a much higher degree in warm than cold periods of the year:

Oyster: means of all B-BOMs at LOOP station's for date

	(Zn, ppm)	(Cu, ppm)	(Cd, ppm)
December 1979	389	4.10	.38
June 1980	2,186	23.40	.92
November 1980	532	8.75	.93

This reinforces the assertion made by TerEco at an earlier time that data derived from warm and cold water periods are an absolute necessity to prevent the drawing of invalid conclusions that can be of considerable consequence.

There is a little evidence that test organisms are accumulating metals within their tissues during the 72-hour period of exposure. This finding applies particularly to the oyster and to a lesser extent to Fundulus, and only to a limited number of stations. As shown in Table 8, the average metal concentrations derived from all stations were not significantly

TABLE 8

Means of Trace Metal Concentrations (ppm) in Muscle Tissue
of Test Organisms Held in P-BOMs and B-BOMs
LOOP Survey No. 4 Compared with LOOP Survey No. 3
(Numbers in parentheses are values for No. 4 reference controls)

	Cadmium		Zinc		Copper	
	P-BOM	B-BOM	P-BOM	B-BOM	P-BOM	B-BOM
Fundulus						
Survey No. 3	.07 ± .005	.06 ± .001	106.10 ± 44.94	94.63 ± 4.80	1.85 ± .29	1.50 ± .57
Survey No. 4	.21 ± .22	.30 ± .21	71.50 ± 13.03	65.00 ± 30.66	1.73 ± .40	1.51 ± .80
Ref. Control No. 4	(.24 ± .09)		(63.36 ± 11.44)		(2.25 ± .25)	
Oyster						
Survey No. 3	1.30 ± .14	.92 ± .22	2114 ± 465	1758 ± 421	27.48 ± 12.10	20.33 ± 6.34
Survey No. 4	No Sample	.93 ± .60	No Sample	464 ± 96	No Sample	8.54 ± 2.91
Ref. Control No. 4	(.61 ± .14)		(532 ± 156)		(8.75 ± 1.48)	
Shrimp (*Penaeus*)						
Survey No. 3	(Anoxic Mortality)		(Anoxic Mortality)		(Anoxic Mortality)	
Survey No. 4	No Sample	.28 ± .19	No Sample	51.50 ± 11.92	No Sample	21.39 ± 3.29
Ref. Control No. 4	(.32 ± .08)		(53 ± 5.66)		(22.60 ± .85)	

TABLE 9

Mean Concentrations (ppm) of Metals in Samples from Particular Stations LOOP 4

Metal (ppm)	Reference Control	Station 3B	Station 4B	Station 5B	Station 6B	Station 8B
OYSTER						
Cd	.61 ± .10	.91 ± .14	1.76 ± .20	1.88 ± .24	.72 ± .01	.14 ± .001
Zn	532 ± 155	415 ± 114	558 ± 178	539 ± 22	512 ± 88	353 ± 25
Cu	8.75 ± 1.62	5.4 ± .71	9.25 ± 5.16	12.05 ± 1.91	7.20 ± .57	5.7 ± .42
FUNDULUS						
Cd	.24 ± .14	.44 ± .01	.40 ± .001	.32 ± .02	.31 ± .001	.56 ± .001
Zn	63.4 ± 11.4	80 ± .00	49.5 ± .01	94.5 ± .01	73 ± 14	87.5 ± 16

These findings were reflected in the data derived from analyses for the enzyme catalase, particularly in the case of the oyster and to a lesser extent for *Fundulus* (see Table 13). In any case, there is no evidence at the present time that would link bioaccumulation of these metals with diffuser output.

different from the value of the reference control except, perhaps for cadmium in the oyster. But some accumulations at particular stations were notably elevated above the reference controls, as shown in Table 9.

SOME RESULTS OF HYDROCARBON ANALYSES

The data generated by Carbon Systems, Inc. on hydrocarbons in tissues of test organisms show that oysters from Station 3 (diffuser) contained substantially higher levels of alkane and aromatic hydrocarbons than those from any other station. The second highest levels for oysters were found in specimens held in B-BOMs at Station 6, but these levels were only about 8 percent of the alkane and 36 percent of the aromatic concentrations in Station 3 oysters (Table 11). Both concentrations, however, were well above those of the reference control oysters, pointing to the probability that the accumulations occurred at the stations involved. It is possible, however, that the particular oysters exposed at these two stations were collected at a different place from the rest and that they were contaminated prior to collection. Since they were stored in clean water and those at Station 6 were put in BOMs the day after placement of the Station 3 BOM they would have had an additional 24 hours to depurate. In any event, the uptake of hydrocarbons appears to have had little effect on the health of the oysters because their cytochrome P-450/420 levels were moderate (see Metabolic Enzymes in this report).

Unlike the oyster, shrimp and *Fundulus* from Stations 3B and 6B did not exhibit increases in either alkanes or aromatics. The reason for this difference is not known, but it may be related to the fact that whole oysters were analyzed whereas only muscle tissues of shrimp and *Fundulus* were used for hydrocarbon extraction.

Indications are that the oysters in particular were contaminated with refined petroleum hydrocarbons. Whether or not diesel fuel, for instance, can be contained in the brine discharge should be investigated.

TABLE 10

Alkane and Aromatic Hydrocarbon Concentrations (ppm) in Whole Oysters and Muscle Tissue of *Fundulus* and Brown Shrimp Modified from Carbon Systems Report (Appendix B) LOOP Survey No. 4. RF = Reference Control

Station	Alkanes			Aromatics		
	Oysters,	Fundulus,	Shrimp	Oysters,	Fundulus,	Shrimp
1	.42	.37	.08	35.53	2.33	1.07
2	.36	.23	.07	41.97	1.60	.81
3P	---	.29	---	---	2.10	---
3B	6.65	.25	.03	219.33	2.20	.86
4	.22	.26	.02	23.40	1.77	.86
5	.36	.22	.01	38.93	2.20	.63
6	.54	.27	.05	78.83	1.30	1.60
7	.32	.20	.01	37.07	1.53	1.07
8P	---	.23	---	---	1.83	---
8B	.30	.20	.00	25.87	1.40	.77
RF	.35	.34	.02	22.80	2.94	.69

ADENYLATE ENERGY CHARGE

The adenylate energy charge ratio (E.C.) was determined on both the grass shrimp (Palaemonetes pugio) and the brown shrimp (Penaeus aztecus). The results of the laboratory analyses are shown in Table 11 along with the results obtained from Surveys 1, 2, and 3. Several important points can be made:

(1) The E.C. of the grass shrimp averaged higher than during any of the previous surveys. This is particularly important in view of the fact that brine discharges have been a regular occurrence since April 1980. In fact, brine of salinities of about 208°/oo from Cavern 8 and 179°/oo from Cavern 15 was being discharged throughout the period of Survey 4.

(2) The E.C. of grass shrimp held in both the P-BOM and B-BOM deployed at the Diffuser Station 3 were very high, averaging almost as high as those of grass shrimps in the P-BOM and B-BOM at Control Station 8. It is important to note that dissolved oxygen concentrations in both surface and bottom waters were normal in Survey 4.

(3) The above findings make it clear that the high mortality suffered by test grass shrimp during Survey 3 was not caused by brine discharge. As noted elsewhere, the cause was very likely low oxygen in the bottom water - a probability that is strengthened by the observation that grass shrimp in the P-BOMs of Survey 3 did not die (Table 9). Dissolved oxygen concentrations in the surface waters circulating through the P-BOMs were normal at the time.

(4) The E.C. of the brown shrimp also averaged higher than during the previous surveys. Also, those in the B-BOM at the diffuser had the same E.C. as those at the control station.

(5) The high E.C.s of the brown shrimp in the present survey (they were in B-BOMs only) and the normal dissolved oxygen concentrations in the bottom water support the conclusion that the 100% mortality of brown shrimp during Survey No. 3 was caused by anoxia (Table 11).

TABLE 11

Mean Comparison of Adenylate Energy Charge Ratios Between Surveys 1, 2, 3, and 4 (Oct., Dec. 1979; June, Nov. 1980) for Whole Grass Shrimp and Abdominal Muscle of Commercial Shrimp Station 3 is Diffuser; Station 8 is Control

Station	Survey 1	Survey 2	Survey 3	Survey 4
	GRASS SHRIMP (15 animals)			
1 (B-BOM)	.75 ± .12	.95 ± .10	Anoxia	.95 ± .02
2 (B-BOM)	.73 ± .12	.90 ± .09	Anoxia	.92 ± .03
3 (P-BOM, diffuser)	.72 ± .07	.79 ± .10	.79 ± .11	.94 ± .04
(B-BOM)			Anoxia	.96 ± .02
4 (B-BOM)	.86 ± .08	.90 ± .15	Anoxia	.95 ± .03
5 (B-BOM)	.82 ± .12	.92 ± .12	Anoxia	.92 ± .04
6 (B-BOM)	.79 ± .11	.85 ± .04	Anoxia	.94 ± .06
7 (B-BOM)	.84 ± .13	.96 ± .12	.10 ± .001	.91 ± .04
8 (P-BOM)	.77 ± .12	.80 ± .08	.73 ± .11	.98 ± .02
(B-BOM)	.73 ± .17	.85 ± .11	.58 ± .01	.95 ± .03
Reference Control	.77 ± .12	.75 ± .07	.76 ± .18	.93 ± .02
Mean of Means	.78	.86	.60	.94
	COMMERCIAL SHRIMP (6 replicates)			
1	.81 ± .08	.90 ± .9	Anoxia	.85 ± .02
2	.85 ± .14	.93 ± .08	Anoxia	.81 ± .04
3 (B-BOM)			Anoxia	.92 ± .04
4	.83 ± .09	.90 ± .13	Anoxia	.91 ± .07
5	.83 ± .10	.81 ± .07	Anoxia	.86 ± .08
6	.87 ± .01	.93 ± .17	Anoxia	.89 ± .06
7	.78 ± .11	.89 ± .05	Anoxia	.91 ± .05
8 (B-BOM only)	.77 ± .07	.83 ± .05	Anoxia	.92 ± .05
Reference Control	.77 ± .13	.99 ± .07	.94 ± .05	.98 ± .01
Mean of Means	.81	.90		.89

METABOLIC ENZYMES

CYTOCHROME P-450/P-420

As shown in Table 12 there is some evidence of moderate contamination of both the benthic and pelagic environments of this region by petroleum hydrocarbons. The lack of mortality in test species indicates that the levels of contamination are not sufficient to cause acute impacts with the 72-hour test period. Also, the high level of the energy charge seems to indicate that the test species were not suffering stress during the test period.

TABLE 12

Mean Cytochrome P-450/420 Levels in Fundulus (liver), Oyster (gill), and Shrimp (abdominal muscle) Exposed in P-BOMs and B-BOMs
LOOP Survey No. 4, November 1980

Station Number	Cytochrome P-450/420, nanomoles P-450/mg protein				
	Fundulus		Oyster	Shrimp	S
1	.208 ± .03	.100 ± .01	.063 ± .02	.079 ± .04	.077 ± .03
2	.310 ± .04	.149 ± .02	.035 ± .01	.029 ± .02	.027 ± .0
3P	.380 ± .04	.211 ± .04			
3B	.380 ± .08	.126	.023 ± .01	.044 ± .03	.046 ± .02
4	.190 ± .02	.073	.059 ± .01	.05 ± .02	.036 ± .01
5	.406 ± .04	.232	.091 ± .001	.041 ± .02	.051 ± .02
6	.190 ± .04	.114	.012 ± .001	.038 ± .01	.121 ± .02
7	.170 ± .04	.068	.009 ± .0001	.041 ± .02	.049 ± .02
8P	.542 ± .21	.325			
8B	.320 ± .04	.137	.024 ± .0001	.1124 ± .07	.223 ± .0
RF	.590 ± .15	.202	.034 ± .0001	.050 ± .01	.029 ± .01

THE ENZYME ATPASE

The ATPase levels (Table 13) found in Fundulus, the oyster, and shrimp indicate that there were moderate levels of some biphenyl in the water and/or sediments at the time of the test. This might not mean that PCBs or some other biphenyl had been introduced into the area since Surveys 2 and 3 but that such materials were resuspended as a result of the high turbulence before and during the period of Survey 4. Divers deploying and recovering the BOMs reported extreme turbidity.

TABLE 13

Mean ATPase in Fundulus (liver), Oyster (gill), and Shrimp (muscle)
Exposed in P-BOMs and B-BOMs
LOOP Survey No. 4, November 1980

	ATPase Units* x 10^{-4}/mg protein				
Number	Fundulus		Oyster	Shrimp	
1	3.73 ± 1.42	3.47	3.28 ± .37	10.04 ± 2.34	8.81
2	3.41 ± .65	4.94	2.35 ± .29	8.00 ± 1.36	8.27
3P	4.93 ± .66	6.05			
3B	4.36 ± 1.94	4.52	2.67 ± .52	5.93 ± 1.76	8.19
4	3.31 ± 1.01	4.55	3.18 ± .11	7.03 ± 1.12	7.09
5	4.26 ± 1.44	4.35	3.07 ± .65	6.01 ± 1.23	7.06
6	3.50 ± .71	4.42	2.19 ± .21	8.30 ± 2.10	8.99
7	2.94 ± .77	3.58	2.39 ± .44	6.90 ± .58	7.44
8P	5.10 ± .70	4.93			
8B	3.85 ± .17	4.16	1.87 ± .35	6.03 ± 1.55	7.42
RF	4.55 ± .34	3.56	2.68 ± .28	6.46 ± 1.18	6.42

*Bergmeyer Unit for ATPase = amount of enzyme needed to decompose 1 g of NADA (nicotinanide adenine dinucleiotide reduced form) in 1 minute.

THE ENZYME CATALASE

As stated in this report's section on metal analyses, there is some evidence that the oyster and Fundulus are accumulating metals in their tissues during the short period of the test. These findings are reflected to a moderate extent by the catalase levels found in both species at a few of the stations (Table 14). Any evaluation of the impact, if any, caused by this accumulation would require much longer periods of exposure, because the energy charge and mortality data reveal that there are no acute effects.

TABLE 14

Mean Catalase in Fundulus (liver), Oyster (gill), and Shrimp (muscle) Exposed in P-BOMs and B-BOMs LOOP Survey No. 4, November 1980

		Catalase Units* x 10^{-2}/mg protein		
Number		Fundulus	Oyster	Shrimp
1	1.64 ± .57	1.30 ± .30	.285 ± .02	below detection level
2		1.59 ± .30	.339 ± .07	below detection level
3P		1.16 ± .16		
3B		1.06 ± .16	.400 ± .15	below detection level
4		1.82 ± 1.05	.441 ± .11	below detection level
5		1.58 ± .37	.414 ± .03	below detection level
6		1.80 ± .20	.265 ± .03	below detection level
7		1.20 ± .10	.274 ± .01	below detection level
8P		1.38 ± .16		
8B		1.16 ± .56	.770 ± .08	below detection level
RF		1.42 ± .50	.510 ± .12	below detection level

V. SUMMARY CONCLUSIONS

1. LOOP Survey No. 4 was carried out by TerEco and LOOP personnel during the period from 17 to 23 November 1980. Eight B-BOMs and two P-BOMs were deployed at eight sampling stations. The B-BOMs were loaded with Fundulus, Crassostrea, Palaemonetes, and Penaeus; the P-BOMs carried only Fundulus and Palaemonetes.

2. There was no mortality in the test species placed in P-BOMs. Mortality was low in the B-BOMs except for the oyster. Even so, it was less than half what it was in Survey No. 3. The 20 percent November mortality is attributed to the poor condition of the oysters prior to the test and the very heavy siltation observed during the test. It is important to note that little mortality occurred at Station 3 (diffuser); rather the highest oyster mortality was at Station 8 (the control station).

3. The results of the present survey support the conclusion that the heavy mortality found in B-BOMs of Survey 3 (June 1980) resulted from dissolved oxygen deficiency in bottom waters. Salinity increases, which were moderate, had nothing to do with the mortality situation. Thus, whereas the salinities of Surveys 3 and 4 were essentially the same, the dissolved oxygen levels of Survey 4 were well above those of Survey 3.

4. Hydrographic conditions during Survey No. 4 appeared to be normal. The temperature of both surface and bottom waters were well below those of June and well above the lows observed in December. As noted above, salinities were essentially the same as observed in June. Oxygen levels had returned to normal.

5. The adenylate energy charge ratios (E.C.) were excellent. The grass shrimp average from all stations was the highest this far observed in any of the surveys. The E.C. of the brown shrimp was about equal to

the highest observed (Survey No. 2) and well above that of Survey No. 1.

6. Trace metals showed the anticipated seasonal decline in concentration in the tissues of test organisms. For example, zinc in the oyster declined to a November average of 532 ppm from the 2186 ppm in June. In December 1979 the concentration reached an average low of 389 ppm when the bottom water temperature was 16.25°C as compared with the present 19.62°C.

7. There was some evidence that test organisms, particularly the oyster and Fundulus, are accumulating cadmium and zinc in their tissues, even during the 72-hour exposure period. Catalase levels at some stations reflected this uptake. However, there is no evidence that there is any stress accompanying this uptake, since the E.C. ratio is high in all cases.

8. Data generated by Carbon Systems showed that oysters from Stations 3 and 6 contained substantially higher levels of alkane and aromatic hydrocarbons than oysters from any other station. Such increases were not noted in either Fundulus or brown shrimp. There was evidence from cytochrome P-450 that there is moderate contamination of both the benthic and pelagic environments of this region by petroleum hydrocarbons. The source of the hydrocarbons is unknown.

9. ATPase analysis indicated that there was moderate contamination of the pelagic and benthic environments with some biphenyls of unknown type.

10. Evidence at hand indicates that up to the present time the brine discharge at Station 3 has not exerted any unfavorable impact upon either the adjacent pelagic or benthic aspects of the marine ecosystem.

APPENDIX A

WATER CHEMISTRY DATA

LOOP SURVEY 4

DECEMBER 1980

SHILSTONE ENGINEERING TESTING LABORATORY, INC.

ATLANTA, GEORGIA	BATON ROUGE, LA	MONROE, LA	NEW ORLEANS, LA	BEAUMONT, TEXAS	FREEPORT, TEXAS	HOUSTON, TEXAS
ZIP CODE 30306	ZIP CODE 70802	ZIP CODE 71201	ZIP CODE 70112	ZIP CODE 77701	ZIP CODE 77541	ZIP CODE [illegible]
600 VIRGINIA AVE., N.E.	1068 NEOSHO ST.	315 N. SECOND ST.	814 CONTI ST.	2276 PARK ST.	415 NORTH AVENUE F	1714 MEMORIAL DR.
PHONE (404) 872-0795	PHONE (504) 387-3149	PHONE (318) 387-2327	PHONE (504) 524-8306	PHONE (713) 838-1614	PHONE (713) [illegible]	PHONE (713) 224-[illegible]

TESTED FOR: LOOP, Inc
P.O. Box 1159
Harvey, LA 70058
ATTN: Mr. A.J. Heikamp, JR.

PROJECT: Water Analysis

DATE: December 11, 1980

OUR REPORT NO.: 253-03200-37

REMARKS:

DATE RECEIVED:

November 19, 1980

SAMPLE DESCRIPTION:

Bom Sites 1, 3, 8--- 11/18/80

ANALYSIS REQUIRED:

Dissolved Oxygen, Nitrate, Ammonia, Total Kjeldahl, Sulfate, Chloride, Calcium, Total Alkalinity, Phenolphthalein Alkalinity, Ortho-Phosphate, Total Phosphate, Dissolved Solids, Total Suspended Solids, Total Solids, Silicon, Salinity, Turbidity.

METHODOLOGY EMPLOYED:

As per Standard Methods 14th Edition.

RESULTS:

As tabulated below in table form.

sh

Respectfully Submitted,

Shilstone Engineering Testing Laboratory, Inc.

LOOP, Inc.
Water Analysis:

File No.: 2[illegible]-03200-37

Sample Identification	Dissolved Oxygen ppm	Nitrate as N, ppm	Ammonia as N, ppm	Total Kjeldahl Nitrogen	Sulfate as SO_4 ppm	Chloride as Cl ppm	Calcium as $CaCO_3$ ppm	Total Alkalinity as $CaCO_3$ ppm
Bom Site 1 Top 11/18/80	5.9	*ND	0.9	1.2	2568	18,700	388	138
Bom Site 1 Bottom 11/18/80	5.5	*ND	0.4	2.2	2658	19,000	404	136
Bom Site 3 Top 11/18/80	6.3	0.1	0.5	2.0	2691	18,900	400	140
Bom Site 3 Bottom 11/18/80	6.5	*ND	0.8	1.4	2551	18,900	388	134
Bom Site 8 Top 11/18/80	6.0	*ND	0.6	1.8	2658	18,400	394	138
Bom Site 8 Bottom 11/18/80	6.5	*ND	1.8	1.4	2411	18,400	435	138

*ND = None Detected less than 0.1 ppm

Sample Identification	Phenolpthalein Alkalinity as $CaCO_3$ ppm	Ortho-Phosphate as PO_4 ppm	Total Phosphate as PO_4 ppm	Total Dissolved Solids ppm	Total Suspended Solids ppm
Bom Site 1 Top 11/18/80	20	**ND	**ND	38,345	55
Bom Site 1 Bottom 11/18/80	16	**ND	**ND	38,354	46
Bom Site 3 Top 11/18/80	14	**ND	**ND	37,957	43
Bom Site 3 Bottom 11/18/80	20	**ND	**ND	38,153	47
Bom Site 8 Top 11/18/80	20	**ND	**ND	39,755	45
Bom Site 8 Bottom 11/18/80	20	**ND	**ND	38,957	43

**ND = None Detected less than 0.1 ppm

Sample Identification	Total Solids ppm	Silicon as SiO_2 ppm	Salinity o/oo	Turbity as SiO_2 ppm
Bom Site 1 Top 11/18/80	38,400	1	32.32	14
Bom Site 1 Bottom 11/18/80	38,400	2	32.75	3
Bom Site 3 Top 11/18/80	38,000	1	32.61	11
Bom Site 3 Bottom 11/18/80	38,000	2	32.61	15
Bom Site 8 Top 11/18/80	39,800	1	31.65	17
Bom Site 8 Bottom 11/18/80	39,000	1	31.63	25

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SHILSTONE ENGINEERING TESTING LABORATORY, INC.

ATLANTA, GEORGIA ZIP CODE 30306 0 VIRGINIA AVE., N.E. PHONE (404) 872-0795	BATON ROUGE, LA ZIP CODE 70832 1068 NEOSHO ST PHONE (504) 387-3149	MONROE, LA ZIP CODE 71201 315 N. SECOND ST PHONE (318) 387-2327	NEW ORLEANS, LA ZIP CODE 70112 814 CONTI ST PHONE (504) 524-8395	BEAUMONT, TEXAS ZIP CODE 77701 2276 PARK ST PHONE (713) 838-1694	FREEPORT, TEXAS ZIP CODE 77541 415 NORTH AVENUE F PHONE (713) 233-6366	HOUSTON, TEXAS ZIP CODE 77007 1714 MEMORIAL DR PHONE (713) 224-2547

TESTED FOR: Loop, Inc.
P.O. Box 1159
Harvey, LA 70058
ATTN: Mr. A.J. Heikamp

PROJECT: Water Analysis--Special Study

DATE: December 11, 1980

OUR REPORT NO.: 253-03200-38

REMARKS: DATE RECEIVED:

November 20, 1980

ANALYSIS REQUIRED:

Dissolved Oxygen, Nitrate, Ammonia, Total Kjeldahl, Sulfate, Chloride, Calcium, Total Alkalinity, Phenolphtein Alkalinity, Ortho-Phosphate, Total Phosphate, Dissolved Solids, Total Suspended Solids, Total Solids, Silicon, Salinity, Turbidity.

METHODOLOGY EMPLOYED:

As per Standard Methods 14th Edition.

RESULTS:

As tabulated below in table form

Respectfully Submitted,

sh

Sample Identification	Dissolved Oxygen ppm	Ortho-Phosphate as PO_4 ppm	Total Phosphate PO_4 ppm	Total Suspened Solids ppm	Total Dissolved Solids ppm	Total Solic ppm
Bom Site 2 Top 11/19/80	6.8	*ND	*ND	16	41,804	41,82
Bom Site 2 Bottom 11/19/80	6.5	*ND	*ND	10	40,570	40,58
Bom Site 4 Top 11/19/80	6.4	*ND	*ND	13	45,027	45,04
Bom Site 4 Bottom 11/19/80	6.4	*ND	*ND	41	59,639	59,68
Bom Site 5 Top 11/19/80	6.4	*ND	*ND	21	42,179	42,20
Bom Site 5 Bottom 11/19/80	6.0	*ND	*ND	32	40,528	40,56
Bom Site 6 Top 11/19/80	6.6	*ND	*ND	28	41,832	41,86
Bom Site 6 Bottom 11/19/80	6.4	*ND	*ND	11	42,369	42,38
Bom Site 7 Top 11/19/80	6.7	*ND	*ND	8	59,852	59,80
Bom Site 7 Bottom 11/19/80	6.5	*ND	*ND	13	39,747	39,70

*ND----None Detected less than 0.2 ppm

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LOOP, INC.
Water Analysis

Sample Identification	Silica as Si ppm	Salinity 0/00	Nitrate as N ppm	Ammonia as N ppm	Total Kjeldahl Nitrogen ppm	Sulfate as SO_4 ppm	Chloride as Cl ppm	Calcium as $CaCO_3$ ppm
Bom Site 2 Top 11/19/80	1	34.40	less than 1	1	0.1	2551	19,989	416
Bom Site 2 Bottom 11/19/80	1	34.30	less than 1	0.6	0.1	2592	19,989	376
Bom Site 4 Top 11/19/80	1	34.52	less than 1	0.2	6	2634	20,059	400
Bom Site 4 Bottom 11/19/80	1	34.40	less than 1	0.6	0.6	2576	19,989	392
Bom Site 5 Top 11/19/80	1	34.40	1	0.5	0.5	2362	19,989	478
Bom Site 5 Bottom 11/19/80	2	34.16	2	0.3	0.4	2527	19,848	439
Bom Site 6 Top 11/19/80	1	34.52	less than 1	*ND	0.4	2510	20,059	376
Bom Site 6 Bottom 11/19/80	1	34.52	1	*ND	0.2	2601	20,059	376
Bom Site 7 Top 11/19/80	1	34.52	less than 1	*ND	0.8	2271	20,059	408
Bom Site 7 Bottom 11/19/80	2	34.52	1	*ND	0.2	2601	20,059	431

* ND -- None Detected less than 0.1 ppm

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Sample Identification	Total Alkalinity as $CaCO_3$ ppm	Turbidity as SiO_2 ppm
Bom Site 2 Top 11/19/80	127	7
Bom Site 2 Bottom 11/19/80	125	8
Bom Site 4 Top 11/19/80	126	15
Bom Site 4 Bottom 11/19/80	125	27
Bom Site 5 Top 11/19/80	127	7
Bom Site 5 Bottom 11/19/80	147	33
Bom Site 6 Top 11/19/80	124	6
Bom Site 6 Bottom 11/19/80	126	7
Bom Site 7 Top 11/19/80	125	8
Bom Site 7 Bottom 11/19/80	126	10

SHILSTONE ENGINEERING TESTING LABORATORY, INC.

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1916 TILLERY ST
PHONE (512) 477 3738

BEAUMONT, TEXAS
ZIP CODE 77705
2480 W. CARDINAL DR. #2
PHONE (713) 842 1020

CORPUS CHRISTI, TEXAS
ZIP CODE 78416
810 S. PADRE ISLAND DR
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1714 MEMORIAL DR
PHONE (713) 224 2047

LAREDO, TEXAS
ZIP CODE 78041
2908 SAN BERNARDO
PHONE (512) 727 3702

SAN ANTONIO, TEXAS
ZIP CODE 78216
8430 WESTERN BLVD
PHONE (512) 342 9377

VICTORIA, TEXAS
ZIP CODE 77901
4021 [illegible]
PHONE (512) 575 0281

TESTED FOR: Loop, Inc.
P.O. Box 1159
Harvey, LA 70058
Attn: Mr. A. J. Heikamp, Jr.

PROJECT Water Analysis

DATE: December 16, 1980

OUR REPORT NO.: 253-03200-39

REMARKS:

DATE RECEIVED:

November 26, 1980

SAMPLE IDENTIFICATION:

Bom Sites 1, 2, 3, 4, 5, 6, 7, 8

ANALYSIS REQUIRED:

Dissolved Oxygen, Nitrate, Ammonia, Total Kjeldahl, Sulfate Chloride, Calcium, Total Alkalinity, Phenolphthalein Alkalinity, Ortho-Phosphate, Total Phosphate, Dissolved Solids, Total Suspended Solids, Total Solids, Silicon, Salinity, Turbidity

METHODOLOGY EMPLOYED:

As per Standard Methods 14th Edition

RESULTS:

As tabulated below in table form

jc

WATER ANALYSIS:

SAMPLE IDENTIFICATION	DISSOLVED OXYGEN PPM	NITRATE AS N, PPM	AMMONIA AS N, PPM	TOTAL KJELDAHL NITROGEN PPM	SULFATE AS SO_4 PPM	CHLORIDE AS Cl PPM	CALCIUM AS $CaCO_3$ PPM	TOTAL ALKALINITY AS $CaCO_3$ PPM
Bom Site #1 Top 11/21/80	6.9	*ND	0.1	0.4	2666	18,300	376	125
Bom Site #1 Bottom 11/21/80	6.6	*ND	0.9	0.4	2724	18,600	384	126
Bom Site #2 Top 11/22/80	7.0	*ND	*ND	0.4	2658	18,400	384	125
Bom Site #2 Bottom 11/22/80	7.4	*ND	0.1	0.3	2708	18,300	353	124
Bom Site #3 Top 11/21/80	6.8	*ND	*ND	0.8	2666	18,500	361	125
Bom Site #3 Bottom 11/21/80	6.7	*ND	1.3	0.7	2658	18,600	376	126
Bom Site #4 Top 11/22/80	6.6	*ND	1.1	1.2	2601	18,300	376	126
Bom Site #4 Bottom 11/22/80	7.3	*ND	*ND	1.4	2658	18,500	376	126
Bom Site#5Top 11/22/80	7.2	*ND	*ND	0.7	2642	18,500	376	126
Bom Site #5 Bottom 11/22/80	7.3	*ND	*ND	0.3	2658	18,300	368	126
Bom Site #6 Top 11/22/80	7.4	*ND	*ND	1.2	2666	18,400	392	122
Bom Site #6 Bottom 11/22/80	7.2	*ND	1.1	0.4	2757	18,500	392	124
Bom Site #7 Top 11/22/80	7.1	*ND	*ND	0.8	2675	18,500	408	126

Continues

WATER ANALYSIS:

SAMPLE IDENTIFICATION	DISSOLVED OXYGEN PPM	NITRATE AS N, PPM	AMMONIA AS N, PPM	TOTAL KJELDAHL NITROGEN PPM	SULFATE AS SO_4 PPM	CHLORIDE AS Cl PPM	CALCIUM AS $CaCO_3$ PPM	TOTAL ALKALINITY AS $CaCO_3$ PPM
Bom Site #7 Bottom 11/22/80	7.2	*ND	*ND	0.4	2666	18,500	392	126
Bom Site #8 Top 11/21/80	7.4	*ND	0.2	1.2	2428	17,800	376	130
Bom Site #8 Bottom 11/21/80	7.1	*ND	*ND	0.6	2551	18,000	392	124

*ND=NONE DETECTED LESS THAN 0.1 PPM

WATER ANALYSIS

SAMPLE IDENTIFICATION	PHENOLPHTHALEIN ALKALINITY AS $CaCO_3$ PPM	ORTHO-PHOSPHATE AS PO_4 PPM	TOTAL PHOSPHATE AS PO_4 PPM	TOTAL DISSOLVED SOLIDS PPM	TOTAL SUSPENDED SOLIDS PPM	TOTAL SOLIDS PPM	SILICON AS SiO_2 PPM	SALINITY AS 0/00	TURB AS SiO_2 PPM
Bom Site #1 Top 11/21/80	1	*ND	*ND	40,732	8	40,740	2	31.67	3
Bom Site #1 Bottom 11/21/80	12	*ND	*ND	40,995	5	41,000	2	32.15	13
Bom Site #2 Top 11/22/80	13	*ND	*ND	39,493	7	39,500	1	31.79	2
Bom Site #2 Bottom 11/22/80	14	*ND	*ND	41,336	4	41,340	1	31.56	7
Bom Site #3 Top 11/21/80	18	*ND	*ND	40,812	8	40,820	1	32.03	7
Bom Site #3 Bottom 11/21/80	18	*ND	*ND	42,397	3	42,400	1	32.15	3
Bom Site #4 Top 11/21/80	18	*ND	*ND	41,887	13	41,900	1	31.67	9
Bom Site #4 Bottom 11/22/80	18	*ND	*ND	39,181	39	39,220	2	32.03	12
Bom Site #5 Top 11/22/80	20	*ND	*ND	39,397	23	39,420	3	31.91	**N
Bom Site #5 Bottom 11/22/80	18	*ND	*ND	40,146	74	40,220	1	31.56	35
Bom Site #6 Top 11/22/80	20	*ND	*ND	41,654	6	41,660	2	31.79	4
Bom Site #6 Bottom 11/22/80	20	*ND	*ND	41,532	8	41,540	2	32.03	5

Continues

LOOP, INC.

WATER ANALYSIS

FILE NO.: 253-03200-39

SAMPLE IDENTIFICATION	PHENOLPHTHALEIN ALKALINITY AS $CaCO_3$ PPM	ORTHO-PHOSPHATE AS PO_4 PPM	TOTAL PHOSPATE AS PO_4 PPM	TOTAL DISSOLVED SOLIDS PPM	TOTAL SUSPENDED SOLIDS PPM	TOTAL SOLIDS PP	SILICON AS SiO_2 PPM	SALINITY AS 0/00	TUR AS SiO PPM
Bom Site #7 Top 11/22/80	22	*ND	*ND	40,676	4	40,680	2	31.91	**N
Bom Site #7 Bottom 11/22/80	20	*ND	*ND	40,517	3	40,520	2	32.03	**N
Bom Site #8 Top 11/21/80	22	*ND	*ND	39,273	7	39,280	2	30.75	9
Bom Site #8 Bottom 11/21/80	18	*ND	*ND	40,255	5	40,260	2	31.20	9

*ND = NONE DETECTED, LESS THAN 0.1 PPM

**ND = NONE DETECTED, LESS THAN 1 PPM

APPENDIX B

POST BRINE DISCHARGE HYDROCARBON ANALYSIS
IN ORGANISM

BY

CARBON SYSTEMS, INC.

LOOP SURVEY 4
DECEMBER 1980

CONTENTS

INTRODUCTION

This report contains the results of hydrocarbon analyses of BOM tissue samples taken in 11/80. This experiment represents the second BOM study following brine discharge into the Gulf. The overall objective of this study is to monitor stress in organisms deployed near the LOOP brine diffuser. Our part of the study involves measuring the hydrocarbon stress to which these organisms have been exposed. This report contains a comparison of hydrocarbon concentrations and molecular distribution patterns with previous samples and reference controls.

SUMMARY

1) Oysters from BOM stations 3B (brine diffuser site) and 6B (SE of brine diffuser) exhibited significant levels of petroleum contamination which originated from possibly one source, the brine diffuser site.

2) Shirmp, Fundulus, and the other oyster samples did not differ significantly from either their respective reference controls or previous tissue samples analyzed.

METHODS OF ANALYSIS

A. Extraction

Oysters were rinsed with methanol and homogenized in a blender. Shrimp and Fundulus samples were rinsed wtih methanol and then finely chopped with a razor blade. Homogenization with a blender could not be used on these samples because of their low water content.

A fifteen gram sample of each organism was taken for each station, and two fifteen gram samples were taken for the reference controls. These tissue samples were digested with NaOH and heat (Warner, 1976). The non-saponifiable lipids were then suspended in ethyl ether and separated using silica gel chromatography.

The silica gel-alumina column, with the sample added, was eluted with 1) hexane to obtain the alkane fraction and 2) 20% (v/v) methylene chloride in petroleum ether solution to obtain the aromatic fraction.

B. Analysis

1. Alkanes

The alkane fraction was analyzed with a Perkin-Elmer Sigma 3 Gas Chromatograph equipped with a flame ionization detector and a 30 meter glass capillary column coated with Supelco SP 2100 liquid phase. Alkane hydrocarbons from C_{15}-C_{32} were identified and quantified with an external standard and corrected for hydrocarbons in the procedural blank. An internal standard, 3-Methylheneicosane (Anteiso C_{22}), was added to each sample before extraction to aid in peak identification and calculation of percent recovery.

2. Aromatics

The aromatic fraction was analyzed with a Perkin-Elmer 204A Fluorescence Spectrophotometer. Samples were excited at 265 nm and scanned from 250-450 nm. Peak intensities were measured at 310 nm, converted to ppm and reported as total aromatic hydrocarbons.

C. Computer Print-out

1. Stars by parameters indicates that the denominator was zero.

2. Alkane hydrocarbons that were below limits of detection (0.001 ppm) are reported as zero.

3. Calculation of CPI Low and CPI High

$$\text{CPI (Low)} = \frac{\dfrac{C_{15} + C_{17} + C_{19} + C_{21}}{C_{16} + C_{18} + C_{20} + C_{22}} + \dfrac{C_{15} + C_{17} + C_{19} + C_{21}}{C_{14} + C_{16} + C_{18} + C_{20}}}{2}$$

$$\text{CPI (High)} = \frac{\dfrac{C_{25} + C_{27} + C_{29} + C_{31}}{C_{26} + C_{28} + C_{30} + C_{32}} + \dfrac{C_{25} + C_{27} + C_{29} + C_{31}}{C_{24} + C_{26} + C_{28} + C_{30}}}{2}$$

4. Total alkanes contain C_{15}-C_{32}.

5. Total saturates contain C_{15}-C_{32} plus pristane and phytane.

RESULTS AND DISCUSSION

The results of chromatographic and fluorimetric analyses of tissue samples are shown in Tables 1, 2 and 3. These results compare total alkane and aromatic hydrocarbon concentrations for baseline BOM tissue samples with samples taken 7/80 and 11/80, following brine discharge from Clovelly Dome.

All oyster samples, except those from stations 3B and 6B, exhibited no significant differences in total alkane or total aromatic hydrocarbon concentrations when compared to either the reference controls or previous oyster samples. Oysters from stations 3B contained significantly higher levels of alkane and aromatic hydrocarbons, while station 6B oysters exhibited moderate but elevated levels. The large majority of alkane hydrocarbons in these samples occurred before n-C_{15} (i.e., lower molecular weight than n-C_{15}, see chromatograms). Alkane hydrocarbons lower than nC_{15} were not quantified or identified, since these low molecular weight hydrocarbons are volatile and are partially lost during sample preparation. Their presence in these oyster samples, however, is highly significant. Oysters from 3B also exhibited a different CPI Low (Carbon Preference Index) from the other oysters. CPI Low values ranged from 4.0-5.5 for the other stations, while the sample from station 3 had a CPI Low of 1.6. This is an indication of petroleum uptake, since the carbon preference in 3B oysters has been shifted from a strong odd-carbon preference, typical of natural systems, to almost no preference for either odd- or even-carbon alkanes (i.e., CPI Low = 1) which is typical of petroleum. The CPI Low for oysters from station 6B was 3.4 which is not significantly different from the other oyster samples.

Shrimp and _Fundulus_ samples did not differ from either their respective reference controls or previous baseline samples analyzed for any of the parameters reported. Shrimp and Fundulus from stations 3B and 6B did not exhibit similar alkane and aromatic characteristics as the oysters from these stations.

Oysters from station 3B and to a lesser extent 6B have been contaminated in some manner with refined or gasoline range petroleum hydrocarbons either when deployed in the BOMs or during subsequent handling after the experiment. Station 3B is at the diffuser site, while station 6B is southeast of 3B. It is possible that the oysters from station 3B have been contaminated with diesel fuel that was entrained in the brine discharge. Station 6B oysters have been contaminated from a similar source but to a lesser extent than oysters at station 3B. This may suggest that the diffuser site is the source of the contamination and the prevailing currents at that time transported the diluted hydrocarbons to station 6B.

The presence of contaminated oysters at stations 3B and 6B and the lack of contamination in shrimp and Fundulus from these stations may be the result of a combination of several factors. Some of these factors are the following: physiological differences between these organisms, exposure time, and the amount of petroleum involved. Oysters would be exposed to larger volumes of dissolved and suspended hydrocarbons than shrimp and fish because of the large volumes of water oysters pump through themselves when feeding. Hydrocarbons have been found to be associated with an organism's lipid pool. Oysters, which have a high lipid content, would tend to retain more hydrocarbons at a given exposure level than fish or shrimp which normally have a lower lipid content. Fish and possibly shrimp possess the ability to metabolize hydrocarbons while oysters cannot. Oysters, however, can depurate themselves of unmetabolized hydrocarbons in a "clean environment." Exposed to low levels of oil, fish and shirmp would be able to "rid" themselves of hydrocarbons through metabolic processes, while oysters would tend to accumulate them until the hydrocarbon levels in the environment decreased to some lower level. Another possible factor is that the whole oyster was analyzed (gut, gill, muscle, etc.) while only the muscle tissue of

Fundulus and shirmp was analyzed. Since muscle would be the last tissue to reflect petroleum exposure, more exposure time would be necessary to demonstrate accumulation in fish and shrimp.

Hydrocarbon uptake during BOM deployment rather than contamination during subsequent handling seems the most likely explanation of the elevated levels in oysters. If contamination occurred during handling of the oysters prior to or following the experiment, other stations should have shown similar quality and levels of hydrocarbons.

Table 1. Comparison of baseline alkane and aromatic hydrocarbon concentrations in oysters with samples taken on 7/80 and 11/80.

	Alkanes				Aromatics			
	Baseline				Baseline			
Station	10/79	12/79	7/80	11/80	10/79	12/79	7/80	11/80
RC	0.20	0.20	1.02	0.35	45.73	37.93	33.09	22.80
8B (7)	----	----	0.71	----	----	----	21.67	----
8B	0.24	0.12	0.38	0.30	26.13	34.03	14.47	25.87
8P	----	----	0.81	----	----	----	20.68	----
7B	0.35	0.09	----	0.32	37.92	51.65	----	37.07
6B	0.51	0.07	----	0.54	25.48	41.02	----	78.83
5B	0.63	0.15	1.11	0.36	31.22	36.79	23.63	38.93
4B	0.59	0.18	1.23	0.22	25.42	38.42	28.75	23.40
3B	----	----	----	6.65	----	----	----	219.33
3P	----	----	2.23	----	----	----	29.74	----
2B	0.52	0.14	----	0.36	37.48	41.70	----	41.97
1B	0.52	0.39	2.72	0.42	38.32	35.49	39.88	35.53

Table 2. Comparison of baseline alkane and aromatic hydrocarbon concentrations in shrimp with samples taken on 7/80 and 11/80.

	Alkanes				Aromatics			
	Baseline				Baseline			
Station	10/79	12/79	7/80	11/80	10/79	12/79	7/80	11/80
RC	0.71	0.00	0.08	0.02	3.77	0.54	5.01	0.69
8B	0.68	0.00	----	0.00	3.66	0.46	----	0.77
7B	0.65	0.03	----	0.01	2.08	0.59	----	1.07
6B	0.44	0.08	----	0.05	1.84	1.24	----	1.60
5B	0.61	0.00	----	0.01	3.25	0.93	----	0.63
4B	0.27	0.01	----	0.02	2.18	2.57	----	0.86
3B	----	----	----	0.03	----	----	----	0.86
2B	0.17	0.13	----	0.07	2.21	4.26	----	0.81
1B	0.19	0.04	----	0.08	11.26	4.52	----	1.07

Table 3. Comparison of baseline alkane and aromatic hydro[illegible] with samples taken on 7/80 and 11/80.

	Alkanes				Aromatics			
	Baseline				Baseline			
Station	10/79	12/79	7/80	11/80	10/79	12/79	7/80	11/80
RC	0.30	0.16	0.06	0.34	4.91	0.17	1.38	2.94
8B (7)	----	----	0.05	----	----	----	1.14	----
8B	0.29	0.13	0.13	0.20	4.65	0.43	1.92	1.40
8P	0.29	0.26	0.06	0.23	3.68	0.49	1.33	1.83
7B	0.41	0.11	----	0.20	6.01	0.45	----	1.53
6B	0.38	0.10	----	0.27	4.49	0.39	----	1.30
5B	0.55	0.03	----	0.22	7.49	0.39	----	2.20
4B	0.45	0.19	----	0.26	5.14	0.52	----	1.77
3B	----	----	----	0.25	----	----	----	2.20
3P	1.15	0.25	0.09	0.29	14.40	0.34	1.14	2.10
2B	0.82	0.04	----	0.23	6.18	0.20	----	1.60
1B	0.47	0.02	----	0.37	2.12	0.18	----	2.33